Remarks

Claims 106-114, 116-136, 138-140, 142-145, 147, 157-167 and 172-174 are pending.

Double Patenting:

Claims 107-114, 116-136, 138-140, 142-145, 147, 157-167, and 172-174 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting over claims 119-135 of copending application Serial No. 10/836,856.

Where neither of the co-pending applications has yet been deemed to contain allowable claims, Applicants respectfully request that the rejection be held in abeyance until either application has allowable claims. Upon notice that claims are allowable, Applicants intend to submit a terminal disclaimer.

Rejections under 35 U.S.C. § 103

1. Claims 107-114, 116-136, 138-140, 142-145, 147, 157-167, and 172-174 are rejected under 35 U.S.C. 103(a) based on the combination of Werther et al., Fire et al., Heifetz et al., Calabretta et al., Taira et al., and Thompson et al.. Applicants respectfully disagree with this rejection under 35 U.S.C. § 103(a) for the reasons discussed below.

First, in explaining why Applicants' previous arguments were not persuasive, the Office Action mailed 11/18/2009 states at page 4, paragraph 3, lines 1-4:

> "Heifetz et al. does **in fact** teach expression vectors comprising two or more different double stranded RNA sequences wherein the dsRNA sequences are complementary to target sequences and capable of mediating inhibition of target gene expression." (emphasis added)

However, Applicants note that the Office Action mailed 5/20/2009 states at page 8, paragraph 1, lines 8-12:

> "Heifetz et al. does **not** teach expression vectors comprising two or more different double stranded RNA sequence that are complementary to two or more sequences of at least one mammalian target gene and does not teach said expression vector expresses two different double stranded RNA sequences using two promoters wherein the promoters are RNA pol III promoters." (Emphasis added)

These statements are clearly in conflict with each other, in that the statement in the 5/20/09 Office Action indicates that Heifetz et al. does *not* teach "expression vectors comprising two or more different double stranded RNA sequences..." while the statement in the instant Office Action states that Heifetz et al. *does in fact* teach "expression vectors comprising two or more different double stranded RNA sequences..." Oddly, where these statements are directly contradictory, the instant Office Action does not explain where it is that the Heifetz et al. reference provides the subject disclosure. Applicants respectfully request that the Examiner indicate where the Heifetz et al. specification teaches "expression vectors comprising two or more different double stranded RNA sequences wherein the dsRNA sequences are complementary to target sequences and capable of mediating inhibition of target gene expression" as stated in the Final Office Action. Applicants submit that upon close reading of the Heifetz et al. reference, there is no apparent teaching or suggestion of a multitarget partially double-stranded RNA molecule comprising two or more different double-stranded RNA sequences or a vector encoding such an RNA molecule.

Applicants understand that obviousness cannot be overcome by attacking the references separately. Nonetheless, in articulating the reasoning necessary to properly support a prima facie case of obviousness, the Patent Office must accurately set out the teachings of the prior art relied upon. *KSR Intn'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007); see also *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966). Where Fire and Heifetz et al. are the only references cited that teach the use of dsRNA to modulate gene function, it is critically important that any conclusions based upon those references be based on an accurate understanding of their teachings. Where neither Heifetz et al. nor Fire et al. provide a teaching of a multitarget double-stranded RNA or a vector expressing such an RNA, these references cannot be used to support a conclusion of obviousness based in any way upon such a purported teaching.

In addressing the reasons why the Patent Office believes the claimed invention is obvious over a combination of references including several that only teach ribozymes and antisense RNA, despite the clear differences between these different approaches, the Office Action states:

> There is nothing in the rejection of record that requires the use of the methods of the cited references. *Moreover, it is well known in the art that ribozymes, antisense molecules and siRNA all cleave the target RNA by different mechanisms however it is also well known in the art that each of the molecules require a single stranded antisense sequence that recognizes and binds to the target RNA to mediate cleavage of the target RNA and inhibition of expression which is the common purpose of each of the molecules.* Thus, one of ordinary skill in the art would have found the approach to simultaneous targeting of genes taught by Werther et al. and Calabretta et al. applicable to the claimed RNA molecule. (Emphasis added)

Applicants respectfully disagree.

First, Applicants submit that as of the April 21, 1999 priority date of the instant application or even the April 20, 2000 filing date of the parent international application, the mechanisms of siRNA inhibition were not well understood. In particular, contrary to the assertions in the Office Action, it was *not* known in the art that dsRNA-mediated gene silencing required a single-stranded antisense sequence that recognizes and binds target RNA to mediate cleavage of the target RNA. In fact, the role of the antisense RNA strand of the siRNA in cleavage was not established until 2002. This conclusion is supported by a review of the literature available at the time of invention and thereafter, as well as by the statements made in the Rule 132 Declarations submitted herewith from two experts on RNAi technology: Dr. Rachel Meyers; and inventor Dr. Catherine Pachuk.

The following provides statements from publications regarding dsRNA-mediated gene silencing near that time period that establish that the mechanism of dsRNA-mediated gene silencing, particularly with respect to the role of the antisense strand of an siRNA in the process, was not known prior to the filing of the instant application:

1) The 2004 review article, Bantounas et al., J. Mol. Endocrinol. 33: 545-557, titled "RNA interference and the use of small interfering RNA to study gene function in mammalian systems"

(**Exhibit A**) states, in its abstract, "In this review, **an historic overview of the biochemistry of the RNAi pathway is described** together with the latest advances in the RNAi field." The reference proceeds to describe the steps in the RNAi pathway, stating (page 546, left column):

> The first step in the RNAi pathway involves the processing of large dsRNAs into small, 21-23 nucleotide long siRNA molecules (Zamore et al. 2000, Elbashir et al. 2001). Initial studies in Drosophila showed that an RNAse III enzyme (known to recognize dsRNA and reviewed in Conrad & Rauhut 2002) was responsible for this processing and that the siRNAs possessed 3' hydroxyl and 5' phosphate groups, and importantly, a 3' overhang of two unpaired nucleotides on each strand (Elbashir et al 2001). A specific RNAse III enzyme was then found to be responsible for cleaving the dsRNAs and was named Dicer (Bernstein et al. 2001). ....

The Bantounas et al. review then proceeds to state (p. 546, right column):

> Following the cleavage of dsRNA into siRNAs by Dicer the second important stage of mRNA degradation occurs. This is mediated by a protein complex with nuclease activity known as RISC which is guided to its target RNA by siRNA (Hammond et al. 2000). This guide role of siRNA was proposed after the observation that dsRNA would only lead to the degradation of an mRNA with a homologous sequence, leaving the rest of the RNA in the cell unaffected. Moreover, it was shown that both siRNA and protein were required to mediate cleavage of the target (Hammond et al. 2000). Following the initial discovery of the existence of a ribonucleoprotein complex as a mediator of RNAi, the components and mechanism of action of RISC began to be elucidated and both inactive and active forms of RISC complex (the active termed RISC*) were found. Nykanen et al. (2001) found that a second ATP-dependent step was involved in the pathway and *showed that following unwinding of the siRNA duplex*, RISC was converted to RISC*. **In a separate seminal study, RISC* was found to be associated only with the antisense strand of the siRNA (Martinez et al., 2002)**. (Emphases added)

This explanation of the evolution of the art shows that prior to the study by Nykanen et al. in 2001 (**Exhibit B**), it was not known that RNAi involved unwinding of the two strands of the siRNA. That is, contrary to the assertion in the Office Action, it was not well known before 2001 that siRNA requires a single-stranded antisense sequence. More importantly, prior to the "seminal study" by Martinez et al. **in 2002** (Cell, volume 110: 563-574, **Exhibit C**), it was not generally

understood that only the antisense strand of the siRNA was associated with the active form of the RISC. The introduction of the Martinez et al. reference states (563, right column, last paragraph):

> RISC assembled on siRNA duplexes in D. melanogaster embryo lysate targets homologous sense as well as antisense single stranded RNAs for degradation (Elbashir et al., 2001b, 2001c). The cleavage sites for sense and antisense target RNAs are located in the middle of the region spanned by the siRNA duplex. The 5' end, and not the 3' end, of the guide siRNA sets the ruler for the position of the target RNA cleavage (Elbashir et al., 2001b, 2001c).

That is, Martinez et al. teaches that sense or antisense RNA can be targeted by RNAi. The Martinez et al. reference continues (p. 564, first paragraph):

> Unwinding of the siRNA duplex must occur prior to target RNA recognition. Analysis of ATP requirements revealed that the formation of RISC on siRNA duplexes required ATP in lysates of D. melanogaster (Nykanen et al., 2001). Once formed, RISC cleaves the target RNA in the absence of ATP (Hammond et al., 2000, Nykanen et al., 2001). The need for ATP probably reflects the unwinding step and/or other conformational rearrangements. *However, it is currently unknown if the unwound strands of a siRNA duplex remain associated with RISC or whether RISC only contains a single stranded siRNA.* The symmetric cleavage of sense and antisense target RNA by siRNA duplexes (Elbashir et al., 2001b, 2001c) may be explained by the presence of approximately equal populations of sense and antisense strand containing RISCs. (Emphasis added)

That is, as of the publication of Martinez et al. in 2002, and certainly not before the April 1999 priority date or the April 2000 international parent application filing date, it was not known whether the active form of the RISC contained only the antisense RNA. Note also that the Martinez et al. reference independently corroborates Nykanen et al. as teaching unwinding of the siRNA duplex in 2001. It was not well known before 2001 that RNAi involved unwinding of the dsRNA duplex.

The Martinez et al. reference continues (at p. 564, left column, 3rd paragraph):

> Here, we report on the analysis of human RISC in extract prepared from HeLa cells. The reconstitution of RISC and the mRNA targeting step were exploited for affinity purification of RISC *and revealed the RISC is a ribonucleoprotein complex*

> *that contains a single-stranded siRNA and proteins of the Argonaute family.* Once RISC is formed, the incorporated siRNA can no longer exchange with free siRNAs. ***Surprisingly,*** *RISC can be reconstituted in HeLa S100 extract providing single-stranded RNAs.* ***Even more surprising,*** *single-stranded antisense siRNAs transfected into HeLa cells potently silence an endogenous gene with similar efficiency to duplex siRNA.* (Emphases added)

That is, the "seminal" Martinez et al. reference was the first to confirm that RISC includes only single stranded RNA, in 2002. Further, Martinez et al. characterized it as "surprising" that single-stranded RNA could be assembled into RISC and could silence an endogenous gene in a living cell. Thus, aspects of RNAi that may be taken for granted today were in no way well known in the art as of the 1999 priority date of the instant application or even as of the 2000 filing date of the international parent application. This is fully supported in the accompanying declaration by Dr. Rachel Meyers, an expert with an in-depth and intimate working knowledge of the field of post-transcriptional gene silencing, and particularly in the mechanisms of double-stranded RNA-mediated gene silencing. In her Declaration, Dr. Meyers states, in part:

> The Examiner apparently assumes that because one of skill in the art at the time of the invention knew that each of antisense, ribozyme and siRNA requires a single-stranded antisense sequence that recognizes and binds to the target RNA to mediate cleavage of the target RNA, it would have been obvious to apply the teachings of antisense and ribozyme technologies to the siRNA field where one wished to prepare multitarget dsRNAs. *This assumption is not at all accurate in my opinion*, as discussed below. *Specifically, the particular involvement of the antisense strand in siRNA mediated cleavage was not understood at the time the present application was filed, but was elucidated at a later date after the RNA-induced silencing complex (RISC) was recognized to be involved in post-transcriptional gene silencing.* The RISC complex and its role in gene silencing were not discovered until after the filing date of the present application. A review of the literature regarding the biochemistry of the RNAi pathway clearly supports this position. (Meyers Declaration, ¶10; emphases added)

Dr. Meyers continues, referring specifically to the Nykanen and Martinez references:

> For example, it is clear from publications by Nykanen et al. (Nykanen A. et al., "ATP Requirements and Small Interfering RNA Structure in the RNA Interference Pathway" *Cell* 107:309-321 (2001); Exhibit A) and Martinez et al (Martinez, J. et al. "Single-Stranded Antisense siRNAs Guide Target RNA

> Cleavage in RNAi" *Cell* 110:563-574 (2002); Exhibit B), both of which were published *after* the filing of the present patent application, that although the RISC complex had been recognized to be involved in siRNA mediated gene silencing, the particular interaction of the siRNA with both the RISC complex and the target RNA was still unclear. That is, contrary to the Examiner's assertion, the interaction of an siRNA with the other components of RISC, and the subsequent interaction of the RISC complex with the target RNA was not yet elucidated. This is clear from the Nykanen et al. reference, which recites in the figure legend for Figure 8 that
>
> > "We do not yet know if only one or both siRNA strands are present in the same RISC complex."
>
> *This statement indicates that the mechanism of siRNA-mediated gene silencing was not yet known -that is, it was not yet known whether an siRNA would necessarily mediate gene silencing via the antisense strand. As such, it was* ***not*** *well known that antisense and siRNA each require a single-stranded antisense sequence that recognizes and binds to the target RNA to mediate cleavage of the target RNA as asserted by the Examiner in maintaining the present obviousness rejection.* (Meyers Declaration, ¶11; emphases added)

Thus, Dr. Meyers indicates that as of the filing date of the instant application, the interaction of the siRNA with the RISC and with the target RNA were not understood, and it was not yet known whether an siRNA would necessarily mediate gene silencing via the antisense strand. Dr. Meyers continues:

> To my understanding, the first indication that an antisense strand of a duplex siRNA is involved in dsRNA mediated gene silencing was the paper by Martinez et al.(Martinez, J. et al. "Single-Stranded Antisense siRNAs Guide Target RNA Cleavage in RNAi" *Cell* 110:563-574 (2002)). Martinez et al. report that when they added a single-stranded antisense siRNA into cells an endogenous gene was silenced with similar efficacy to a duplex siRNA. Martinez et al. were surprised by this finding as evidenced by the statement
>
> > "Even more surprising, single stranded antisense siRNAs transfected into HeLa cells potently silence an endogenous gene with similar efficacy to duplex siRNA." (Meyers Declaration, ¶12)
>
> In addition, to my understanding Martinez et al. are the first group to report that only a single strand of siRNA is contained in the RISC complex and that single-stranded antisense siRNA permits target RNA cleavage by the RISC complex. Thus, it was not until the Martinez publication in *Cell* in September 2002 that it

> was demonstrated or known that the antisense strand of a duplex siRNA is necessary for target RNA recognition and cleavage. This recognition of siRNA mechanism of action came several years after the earliest priority date of the present application (i.e, April 21, 1999). (Meyers Declaration, ¶13)

Thus, Dr. Meyers again emphasizes that the role of the antisense strand in dsRNA mediated gene silencing was not known as of the filing of the instant application . Dr. Meyers goes on to state that, in her opinion, " it would therefore not have been safe to assume that an element that applies to antisense or ribozymes would necessarily provide the same function in the siRNA context," and that " it is my opinion that those skilled in the art at the time of the present application did not know, and could not have known, as much as the Examiner assumes." Dr. Meyers concludes by stating that "It appears that the Examiner is using knowledge that is well understood in the field today to form the basis of the rejection, however this knowledge was simply not available to those of skill in the art at the time of filing."

Inventor Dr. Catherine Pachuk, also an expert in the siRNA field, and now a Senior Director at Pfizer Research Technology Center of Pfizer Pharmaceuticals, voices similar conclusions in her Rule 132 Declaration. In particular, she specifically disagrees with the statement in the Office Action that "it is well known in the art that ribozymes, antisense molecules and siRNA all cleave the target RNA by different mechanisms however it is also well known in the art that each of the molecules require a single stranded antisense sequence that recognizes and binds to the target RNA to mediate cleavage of the target RNA and inhibition of expression which is the common purpose of each of the molecules." Dr. Pachuk states:

> It is now well known in the field that the *mechanism of association* with a target RNA is different between antisense/ribozymes and siRNA. Furthermore, the field of post-transcriptional gene silencing using double stranded RNA (dsRNA) molecules was in its infancy at the time of filing and the mechanism of action of a double-stranded RNA effector molecule in mediating target cleavage of an RNA was not yet fully understood. In particular, it was not known that dsRNAs bind to the target mRNA using a single stranded antisense sequence. (Pachuk Declaration, ¶ 6)

Thus, Dr. Pachuk emphasizes that the role of the antisense strand of the dsRNA in double-stranded RNA-mediated gene silencing was not known in the art at the time of filing. Dr. Pachuk continues, noting that "the involvement of the RISC complex for processing small dsRNA effector molecules was not yet known." Dr. Pachuk continues further, stating "It was not until the RISC complex was implicated in the unwinding and cleavage of dsRNA molecules that it was first hypothesized that the antisense strand of a dsRNA molecule binds to target RNA to mediate cleavage" and citing the Elbashir et al. (2001), Nykanen et al. (2001) and Martinez et al. (2002) references (Pachuk Declaration, ¶ 6). She states:

> At the time that the present application was filed, it was not yet known in the art that dsRNA molecules acted through a mechanism whereby the antisense sequence binds to a complementary region of a target RNA to mediate cleavage.

Dr. Pachuk thus concludes:

> It follows then that the Examiner's arguments are based on parts of what are known in the art, at best, at a time after the present application was filed, and at worst, at the *present* time, rather than what was known in the art at the time of filing. (Pachuk Declaration, ¶ 6).

Thus, Dr. Pachuk's Declaration is in agreement with that of Dr. Meyers regarding assumptions apparent in the conclusions drawn in the Office Action's obviousness rejection. Dr. Pachuk explains further, discussing the conceptions of those in the field at the time, stating that as of April 29, 1999:

> **It was not clear how a double stranded siRNA could bind to a single strand RNA**. This is a big difference between antisense RNA and ribozymes which each have single stranded regions that enable binding. *Simple melting of the siRNA could not explain this, because, for many siRNAs, the Tms were too high for duplex melting to be a plausible explanation.* (Pachuk Declaration, ¶ 7; emphases added).

This clear difference in the physical parameters of siRNA relative to the effectors in antisense- and ribozyme-mediated gene silencing therefore raised questions that would not have directed

one of skill in the art to the antisense or ribozyme arts for solutions to the problem of targeting multiple targets with siRNAs. Dr. Pachuk states in this regard:

> In my opinion and in view of the above, one of skill in the art at the time of the invention would not have recognized that ribozymes, antisense and dsRNA each require a single strand of RNA that binds to a complementary region of a target RNA to effect target cleavage. As such, one of skill in the art would not have been motivated to take elements known to work in the ribozyme and antisense literature and apply them to molecules for dsRNA mediated inhibition of gene expression. In particular, one of skill in the art would not have combined the teachings of the Werther et al., Fire et al., Heifetz et al., Calabretta et al., Taira et al., and Thompson et al. references to arrive at the presently claimed invention as proposed by the Examiner. *Further, it was not known that the elements of antisense and/or ribozymes for mediating inhibition of gene expression could be applied together with, or would be amenable to, the generation or activity of siRNA molecules from precursor RNAs.* (Pachuk Declaration, ¶ 8; emphasis added)

While the Office Action acknowledges that the mechanisms of dsRNA-, antisense- and ribozyme-mediated gene silencing are different, the Office Action argues that one of skill in the art would have found the approach to simultaneous targeting of genes taught by Werther et al. and Calabreta et al. (both antisense references) applicable to the claimed RNA molecule. Dr. Pachuk disagrees, stating that in her opinion, the Examiner has:

> not taken into account the knowledge understood in the art at the time of filing, particularly with regard to the mechanism of action of a dsRNA molecule. That is, contrary to the Examiner's assertions in the Office Action, it was not well known in the art at the time that each of antisense-, ribozyme- and dsRNA-mediated inhibition "require a single stranded antisense sequence that recognizes and binds to the target RNA to mediate cleavage of the target RNA." In my opinion then, the reasoning regarding the obviousness conclusion is based on an incorrect assumption regarding what was known in the art at the time.

The declarations of Drs. Meyers and Pachuk, as well as the Nykanen and Martinez papers to which they and the Bantounas et al. review refer, provide ample evidence that it was not "well known in the art" at the time the priority application or the international parent application were

filed that "each of the molecules require a single stranded antisense sequence that recognizes and binds to the target RNA to mediate cleavage of the target RNA and inhibition of expression" as asserted by the Office Action. As such, Applicants submit that the proposed motivation to combine references teaching solely ribozyme and antisense technologies with teachings regarding dsRNA necessarily fails. Because the role of the single antisense strand was not established until after the earliest priority date or even the filing date of the international parent application, it would only be with reference to the instant specification and claims that one might propose the stated motivation to combine the present combination of references. The Court in *KSR* cautioned against hindsight bias, stating:

> "A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning. See Graham, 383 U.S., at 36 (warning against a temptation to read into the prior art the teachings of the invention in issue" and instructing courts to "'guard against slipping into the use of hindsight'" (quoting *Monroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406, 412 (CA6 1964))).

Applicants note that the reasoning expressed in the Office Action with regard to the asserted motivation to apply the teachings of the antisense references Werther et al. and Calabretta et al. in the context of dsRNA resonates in hindsight reconstruction when it states "Thus, one of ordinary skill in the art would have found the approach to simultaneous targeting of genes taught by Werther et al. and Calabretta et al. ***applicable to*** *the claimed RNA molecule*" (emphasis added). Without intending any admission in that regard, Applicants submit that the possibility that the references could be seen as "applicable to" the claimed RNA molecule is not the proper inquiry – rather, the proper question is whether one of skill in the art, given the teachings of Werther et al. and Calabretta et al. would have been motivated to combine the teachings of these antisense references with a reference teaching dsRNA *to arrive at the claimed RNA molecule*, and to do so *without* the benefit of Applicants' disclosure.

Claims 107-114, 116-136, 138-140, 142-145, 147, 157-167 and 172-174 remain rejected as obvious under 35 U.S.C. §103(a) over Taira et al. in view of Fire et al. and Thompson et al. The

Office Action discusses the teachings of Taira et al. and Thompson et al. only in the context of Heifetz et al., which is not part of the instant rejection. Specifically, the Office Action states, on page 5:

> In response to Applicant's arguments regarding Taira et al. and Thompson et al., Heifetz et al., while teaching vectors comprising two or more different double-stranded RNA sequence wherein the dsRNA sequences are complementary to target sequences and capable of mediating inhibition of target gene expression do not teach said expression vector expresses two different double stranded RNA sequences using two promoters wherein the promoters are RNA pol III promoters. Taira demonstrates that it was well known in the art to use expression vectors comprising cleavage sequences for expressing multiple RNA molecules that are capable of mediating inhibition of target gene expression. Further, Thompson et al. demonstrates it was well known in the art to incorporate RNA pol III promoter into the expression vector because said promoters are more attractive for expression of RNAs in all tissue types and the accumulation in cells is greater than from a pol III based expression vector. Thus, one of ordinary skill in the art would have found the expression vectors taught by Taira et al. and the use of RNA pol III promoters taught by Thompson et al. applicable to the claimed invention and would have had a reasonable expectation of success at generating an expression vector comprising one or more different RNA molecules.

Applicants respectfully disagree. As discussed previously, Taira et al. teaches only ribozyme technology, and Heifetz et al. does not teach vectors comprising two or more different double-stranded RNA sequences wherein the dsRNA sequences are complementary to target sequences and capable of mediating inhibition of target gene expression.

First, Applicants reiterate the discussion above regarding knowledge in the art at the time of the invention. Contrary to the assertions in the Office Action, and again considering the statements of Drs. Meyers and Pachuk in the ir respective declarations, it was not well known in the art in 1999 or 2000 that ribozymes, antisense and siRNA each require a single stranded antisense sequence that recognizes and binds to the target RNA to mediate cleavage of the target RNA and inhibition of expression. Any motivation to combine teachings on that basis necessarily fails.

Second, Taira et al. describes vectors in which an encoded ribozyme *cleaves itself* out of a primary transcript. Whether there is one, or more than one ribozyme encoded on the primary transcript, the fact is that the ribozyme *cleaves itself* out of the transcript in a monomolecular reaction to generate the active molecule. This is considerably different from the situation in a dsRNA context, in which: a) cutting between different partially double stranded RNAs would, for example, require recognition and cleavage by a separate cleaving enzyme; b) processing of the dsRNA to active siRNA involves a separate, and at that time (1999-2000) poorly understood, enzyme reaction for proper processing to the active 21-23-mer oligonucleotides; and c) the impact of one partially double-stranded RNA molecule being the substrate for *both* the cleaving enzyme that separates the different dsRNAs *and* the enzyme that processes the dsRNAs to siRNAs was not known . Because cleavage between different dsRNAs is not a monomolecular reaction as in the ribozyme context, it was not known, for example, whether Dicer and a given separate cleavage enzyme would interfere with each other, sterically or otherwise. The mechanism and specificity of Dicer cleavage were not known at the time of filing. Thus, even if Taira et al. teaches more than one ribozyme separated by cleavage sequences, it would not have been a foregone conclusion that placing cleavage sequences between different dsRNA sequences, instead of between ribozyme sequences, would permit their proper cleavage *and* processing to siRNAs. Thus, not only would there not have been a motivation, per se, to apply the teachings of Taira et al. to non-ribozyme expression inhibition, there also would not have been a reasonable expectation of success in doing so.

Third, as discussed above, Heifetz et al. does not teach a vector comprising two or more different double-stranded RNA sequences. Not only is this true, but the relevance of Heifetz et al. to a rejection based upon Taira et al., Fire et al. and Thompson et al. is not explained in the Office Action. If the Office Action intended to state that *Fire et al.*, as opposed to Heifetz et al., teaches a vector encoding two or more different double-stranded RNA sequences, this is incorrect as well, because Fire et al. does not.

Finally in this regard, Applicants note that the reasoning expressed in the Office Action with regard to the asserted motivation to apply the teachings of the ribozyme reference Taira et al. in the context of dsRNA reflects hindsight reconstruction when it states "Thus, one of ordinary skill in the art would have found the expression vectors taught by Taira et al. and the use of RNA pol III promoters taught by Thompson et al. ***applicable to*** *the claimed invention*" (emphasis added). Without intending any admission in that regard, Applicants submit that the possibility that the references could be seen as "applicable to" the claimed RNA molecule is not the proper inquiry – rather, the proper question is whether one of skill in the art, given the teachings of Taira et al. (regarding ribozymes and their generation) and Thompson et al. (pol III promoter) would have been motivated to combine the teachings of these references with a reference teaching dsRNA *to arrive at the claimed RNA molecule*, with a reasonable expectation of success, and to do so *without* the benefit of Applicants' disclosure. For the reasons discussed herein above, Applicants submit that that such is not the case.

In summary, at the time of the invention, siRNA technologies were still relatively new, and the mechanism of dsRNA-mediated gene silencing was not clearly understood. Although individual elements of the presently claimed invention might be found in the antisense and ribozyme arts, as discussed above and in the Declarations of Drs. Meyers and Pachuk, it was simply not known or reasonably expected by those of skill in the art at the time of the invention that the combination of features would work in the dsRNA molecule as presently claimed. Therefore, the proposed combination of references cited by the Examiner cannot render obvious the invention as presently claimed.

Conclusion

In view of the reasons discussed above, Applicants respectfully submit that the claims are non-obvious over the cited references for the reasons discussed above. Applicants respectfully request that the rejections under 35 U.S.C. § 103(a) be withdrawn.

In view of the above, all issues raised in the Office Action are addressed herein. Reconsideration of the claims is respectfully requested.

Should any fees be associated with this submission, the Applicants herewith authorize the Commissioner to charge such fees to Nixon Peabody Deposit Account No. 50-0850. Any overpayments should also be credited to said Deposit Account.

Respectfully submitted,

Date: September 20, 2010

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